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BACILLI OF DIFFERENT VIRULENCE

Report II

The Results of Quantitative Calculations

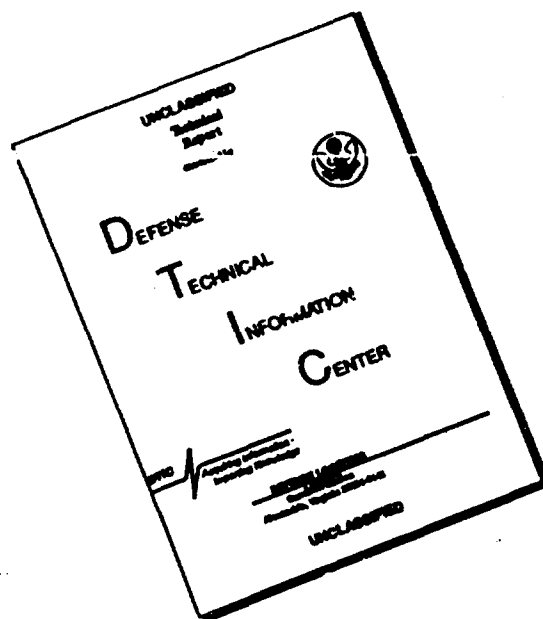
Translation No. 1672

April 1960

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QUANTITATIVE ASSESSMENT OF PHAGOCYTOSIS BY MACROPHAGES IN VITRO OF ANTHRAX  
BACILLI OF DIFFERENT VIRULENCE

Report II

The Results of Quantitative Calculations

[The following is a translation of an article by N. N. Ginsburg and T. N. Maslova, Gamaleya Institute of Epidemiology and Microbiology, AMN, USSR, published in the Russian-language periodical Zhurnal Mikrobiologii Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology), No 1, 1966, Vol 43, pages 125-129. It was submitted on 3 Dec 1964. Translation performed by Sp/7 Charles T. Ostertag, Jr.]

The first report described a method developed by us for the quantitative evaluation of in vitro phagocytosis by macrophages of anthrax bacilli in their vegetative form.\* The mission of the present report was to present the results of a comparative study of the quantitative aspect of phagocytosis by macrophages, obtained from normal and anthrax immune animals, and anthrax bacilli which were avirulent (STI-1) and virulent for guinea pigs (variant 71/12 of the Tsenkovskiy vaccine).

The results of the measurements and calculations made by us are presented in the table, which represents the average values obtained from the processing of 70 sketches. The maximum relative errors in measuring the parameters, which are indicated in the table, did not exceed  $\pm 14\%$ .

The separate phases of the dynamically proceeding phagocytosis are presented in figures 1, 2 and 3. The graphic representations of the main parameters ( $a$ ,  $f$  and  $L$ ), which characterize the reaction under study in the form of functions of time, eased the performance of the comparative analysis of the results of the experiments and made it possible to constitute a notion concerning the course of the entire process.

In comparing the processes of phagocytosis by normal macrophages of virulent (Tsenkovskiy 71/12) and avirulent (STI-1) strains of anthrax bacilli (figure 1), it can be said that they bear a different quantitative and qualitative nature. The reason for this was the different nature of growth of the bacteria (curves  $L$ ) of the virulent and avirulent strains during the period from 3 to 5 hours. For the avirulent strain, after 5 hours from the onset of the test the density of infection of the preparation\*\*

\*J. Microbiol. Epidemiol. & Immunobiol., 1965, No 11, p 124.

\*\*The density of infection ( $L$ ) characterized the overall length (in mm) of the chains of anthrax bacilli per  $1 \text{ mm}^2$  of preparation surface.

turned out to be four times higher than for virulent strain.

The increased activity of the cells (curve  $a$ ) for the STI-1 strain in the initial period (up to 2 hours) was not significant, since it was explained by the fact that the numerous bacilli during the period of initial multiplication have dimensions which are comparable with the dimensions of phagocytic cells. As a result, with a less overall density of infection of the preparation the amount of phagocytized cells was greater. The sharp decline of activity of cells for the STI-1 vaccine during the period from 2 to 5 hours was caused by the rapid increase in the density of infection of the preparation ( $L$ ).

By 5 hours, in spite of the fact that practically all of the cells took part in the process of phagocytosis (curve  $f$ ), parameter  $a$  continued to decrease due to the discontinued intensive growth of bacilli.

Thus the quantity of cells of the culture of macrophages which was selected for the test and the initial dose of its infection with the STI-1 strain did not make it possible to expose changes in the index for the intensity of phagocytosis for this case in a "pure form". In the initial stage of the reaction (up to 2 hours) the value of this parameter was not characteristic, subsequently (from 3 to 5 hours), thanks to the intensive growth of bacteria, the "power" of the cellular defense was completely exhausted, in spite of their 100% participation in the phagocytic reaction. During the period from 2 to 3 hours, when the cells were still able to actively react to an increase in the number of bacteria, curve  $f$  went almost equidistant to curve  $L$  and the index of intensity of phagocytosis for the STI-1 strain and normal macrophages may be roughly taken equal to its average value only during the period from 2 to 3 hours, that is, 45--50 cells per mm.

The virulent Tsenkovskiy 71/12 strain in contrast to the avirulent STI-1 strain multiplied in the presence of normal macrophages somewhat differently, which also determined the nature of the change in all the other parameters. The value of the index of intensity of phagocytosis in this case was not distorted by the dimensions of the bacteria in the initial stage and the shortage of cells in the end stage of the process being studied, as this was noted for the STI-1 strain, and practically remained constant throughout the entire period of observation, with the exception of an insignificant drop after 4 hours. Since the curve for the change of the index of intensity for phagocytosis for the STI-1 strain did not characterize the real value of this parameter, it was not possible to compare the activity of the "invasion" of the macrophages on bacteria of various strains. It can be stated roughly that under equal conditions, when the density of infection of both preparations are the same (point of intersection of curves  $L$  after  $2\frac{1}{2}$  hours), the indices of intensity of phagocytosis for both strains did not differ from each other by more than 20% (45--50 cells for the STI-1 strain and 40 cells per 1 mm for the Tsenkovskiy 71/12 strain).

The discrepancy in the values for the percentage of phagocytosis in this case may be explained by the different density of the preparations.\*

\*"Density of preparation" ( $\rho$ ) - the number of phagocytizing and non-phagocytizing macrophages per 1 mm<sup>2</sup> of preparation surface.

With a comparison of the results of phagocytosis of bacteria of the avirulent STI-1 strain (see figure 2) by normal and immune macrophages, it can be seen that the form of the curves, which characterized the phagocytic reaction in the case of the utilization of immune cells, in general repeated the curves for the changes of parameters  $L$ ,  $a$  and  $f$  for normal cells, but the process of phagocytosis in the first case took place more rapidly with a shift of approximately 2 hours and was practically completed by 5 hours in favor of the macrophages (the density of infection of the preparation was sharply reduced). The process of phagocytosis of bacteria of the avirulent STI-1 strain by normal macrophages over the period of observation (up to 5 hours) was not successfully completed.

The more rapid development of the phagocytic reaction in the system with immune macrophages was explained apparently by the more intensive growth of bacteria (curve  $L$ ) in the period from  $1\frac{1}{2}$  to  $3\frac{1}{2}$  hours. Later the density of infection of the preparation reached the maximum value, which however did not reach the maximum value of the parameter  $L$  for normal cells.

By this moment almost 90% of all the cells took part in the process of phagocytosis (curve  $f$ ), and on the curve for the index of intensity of phagocytosis a characteristic "dip" was observed, which was repeated for normal cells with a delay of  $1\frac{1}{2}$ -2 hours. As it was already pointed out above, the lowering of the index of intensity of phagocytosis for the STI-1 strain was explained by the disparity in the initial doses of the cellular and bacterial populations which were selected for the experiment. This phenomenon turned out to be characteristic both for normal and for immune macrophages. Subsequently therefore, in order to obtain an index of intensity of phagocytosis for the STI-1 strain in a "pure form," it made sense to carry out similar experiments with an increased amount of cells or a reduced dose of the initial bacterial infection.

After a "dip" the intensity of phagocytosis for the immune cells began to rise again, and the density of bacterial contamination was reduced sharply. This indicated that the immune macrophages were rapidly "coping" with the captured chains, simultaneously inhibiting their further growth. All the more free cells or cells with remnants of bacterial chains appeared in a field of vision (curve  $f$  was lowered).

The increase in the index of intensity of phagocytosis for immune macrophages after  $3\frac{1}{2}$  hours again supported the point of view that the lowering of this parameter during the period of maximum density of infection took place due to shortage of cells.

In spite of the relativity of the curves, characterizing the changes in the index of intensity of phagocytosis of bacilli of the STI-1 strain, they practically repeated each other with a lead by 1-2 hours for immune cells. This made it possible to propose that normal and immune macrophages "attack" the avirulent STI-1 strain with the same activity. Here the immune cells enter into the struggle more rapidly than normal cells.

The sectors of the curves up to 2 hours may be disregarded, keeping in mind the great influence of the dimensions of the bacteria with an overall insignificant density of infection of the preparations on the index of the intensity of phagocytosis during this period.

In comparing the results of phagocytosis with normal and immune cells of virulent bacilli of the 71/12 II variant of Tsenkovskiy vaccine (see figure 3, it can be seen that the curves for the change in density of infection of the preparation bore a varied nature. Based on the data of the calculations, the bacteria of the virulent Tsenkovskiy 71/12 strain multiplied 4--5 times more slowly in the presence of immune macrophages. In spite of this, the indices for the intensity of phagocytosis practically coincided during the first 4 hours following the onset of the reaction, comprising on an average of 38--40 cells per 1 mm. This meant, that in the course of this period of time the normal and immune cells "attacked" the chains of the virulent strain with the same activity. The lessening in the intensity of phagocytosis after 4 hours may be explained by the appearance of some factor (for example, a substance discharged by the bacteria), which lowers the activity of the cells and causes their degeneration. This is testified to by the lowering of the density of the preparation (*P*). The influence of this factor was more noticeable for immune cells.

It should be noted that the cited results of the quantitative evaluation of the phagocytic reaction corresponded in the main with the data from our analysis of morphological changes in cells and bacteria. These are the subject of our subsequent reports.

#### Conclusions

1. As a result of the measurements and calculations which were performed, it was established that there were certain peculiarities in the growth and multiplication of bacteria of the STI-1 and Tsenkovskiy 71/12 strains in the presence of normal and immune macrophages.
2. The quantitative evaluation of the results of the tests did not expose any advantage of immune macrophages over normal based on the intensity of phagocytosis, however, in the presence of immune cells there was a more noticeable inhibition of growth of bacteria of both types of strains.
3. The preliminary quantitative results make it possible to propose that immune and normal macrophages of guinea pigs "attack" the bacteria of avirulent and virulent anthrax strains with practically the same activity. This fluctuated within the limits of 35--50 cells per 1 mm.
4. The method of calculations which we developed made it possible to obtain data which objectively characterizes the process of phagocytosis and its dynamic development.



**Main parameters, characterizing the process of phagocytosis of anthrax bacilli by normal and immune cells**

Time (in hours)	STI-1 strain				Tsenkovskiy 71/12 strain							
	f (in %)		a (in cells per 1 mm)	L (in mm <sup>2</sup> /mm <sup>2</sup> )	f (in %)		a (in cells per 1 mm)	L (in mm <sup>2</sup> /mm <sup>2</sup> )				
	Normal	Immune	Normal	Immune	Normal	Immune	Normal	Immune				
0.75	12.25	11.02	79	54	0.41	0.375	11	3.54	47	40	0.94	0.31
1.5	24.8	39.5	86	46	1.01	0.986	18.3	3.8	37	32	1.99	0.567
3	70.5	87	32	16	4.3	14.8	23.2	3.4	38	43	3.3	0.278
5	97.5	44.3	13	42	17.85	0.95	26	6.46	32	24	3.94	0.815
								8.37		14		1.48

Legend:  $f$  - % phagocytosis,  $a$  - index of intensity of phagocytosis,  
 $L$  - density of infection of preparation.

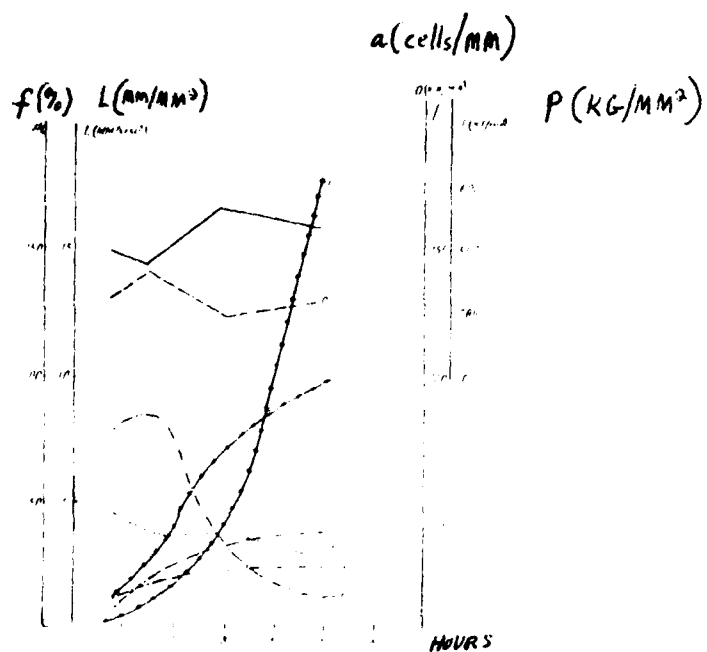


Figure 1. In vitro phagocytosis of bacilli of anthrax from virulent (Tsenkovskiy 71/12) and avirulent strains (STI-1) by normal macrophages.

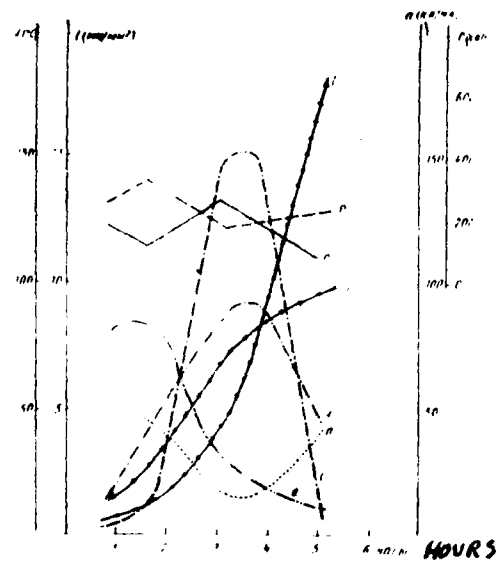


Figure 2. In vitro phagocytosis of anthrax bacilli of the avirulent (STI-1) strain by normal and immune macrophages.

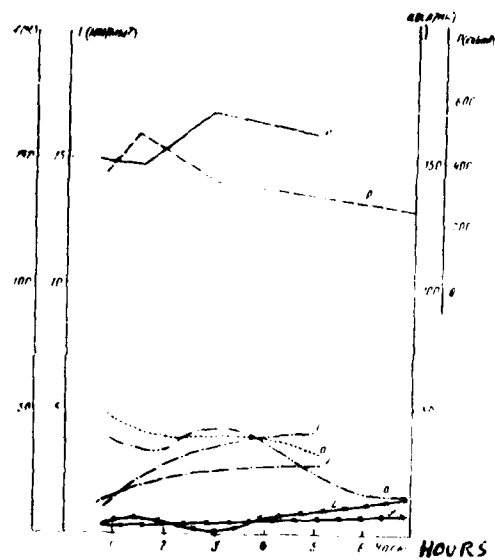


Figure 3. In vitro phagocytosis of anthrax bacilli of the virulent (Tsenkovskiy 71/12) strain by normal and immune macrophages.